Standing out from the crowd: How to identify plasma cells

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Abbreviations: ASC, antibody-secreting cell; Abs, antibodies.

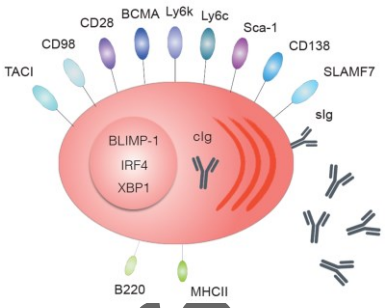
See accompanying articles by Wilmore et al. and Pracht et al.

ABSTRACT

Being the sole source of antibody, plasmablasts and plasma cells are essential for protective immunity. Due to their relative rarity, heterogeneity and the loss of many canonical B-cell markers, antibody-secreting cells (ASCs) have often been problematic to identify and further characterize. In the mouse, the combination of the expression of CD138 and BLIMP-1, has led to many insights into ASC biology, although this approach requires the use of a GFP reporter strain. In the current issue of the *European Journal of Immunology*, two independent studies by Wilmore et al. [Eur. J. Immunol. 2017. 47: XXXX-XXXX] and Pracht et al. [Eur. J. Immunol. 2017. 47: XXXX-XXXX] provide alternative approaches to identify all murine ASCs using antibodies against the cell surface proteins, Sca-1 and TACI, respectively. Here we will discuss the advantages of these new approaches to identify ASCs in the context of our emerging knowledge of the cell surface phenotype and gene expression program of various ASC subsets in the murine and human systems.

Graphical Abstract text

Antibody-secreting plasma cells are a rare lymphocyte population that has proven problematic to identify by flow cytometry. Recently, a number of new markers have been described that enable a more thorough characterization of plasma cells in the murine and human immune systems.



The differentiation of naïve B cells into plasmablasts and plasma cells (collectively termed antibody-secreting cells (ASCs)) is essential for production of the protective antibodies (Abs) that enable us to fight infection. This developmental process also provides immunity against re-exposure with the same infectious agent, through the generation of long-lived memory B cells and plasma cells, and is the basis for virtually all currently used vaccination strategies. Defective ASC development and function results in immune deficiency, while inability to elicit long-lived Ab production is a common limitation of vaccine utility. In contrast, excess or inappropriate Ab production drives the pathology associated with some autoimmune conditions such as systemic lupus erythematosus and Sjögrens syndrome [1] and is an important and chronic complication of organ transplantation [2].

Despite their clear importance for human health, ASCs are a rare cell type that has proven difficult to study and problematic to target therapeutically. This difficulty is due in part to the lack of definitive and universally accepted approaches to identify all ASCs. In this issue of the *European Journal of Immunology*, two studies Wilmore et al. [3] and Pracht et al. [4] have attempted to rectify this situation by identifying and characterising new marker combinations that define ASCs in the mouse. Here we discuss these findings in the broader context of the literature characterising the cell surface phenotype of ASCs in the mouse and the degree to which these insights are translatable to the human immune system.

Part of the difficulty in studying ASCs lies in the lack of high expression of virtually all the antigens used to define the earlier stages of the B-cell lineage. This is due to the unusual genetic control of B-cell terminal differentiation [5] . All stages of committed B cells depend on a core transcriptional program driven by the transcription factor PAX5. PAX5 regulates the expression of many hundreds of genes in B cells including some canonical markers, CD19, CD20 and CD22

(Figure 1A, left panel). Other regulators act as to control gene expression in a more stage specific manner, such as the germinal centre phenotype in the case of BCL6. Critically, the initiation of ASC differentiation results in the silencing of PAX5, BCL6 and many other B-cell regulators which are replaced by a unique ASC gene regulatory network governed by a triad of transcription factors, BLIMP-1 (PRDM1), XBP1 and IRF4 [5]. Thus the expression of all canonical B-cell markers is decreased or lost on ASCs, while a new collection of proteins are expressed that act to facilitate Ab secretion, homing and plasma cell longevity [6].

In keeping with their distinct gene expression program, the uniquely high expression of IRF4, BLIMP-1 and XBP1 identifies all ASCs in mouse and human [6-9]. Indeed, Arumuganaki et al. recently used high IRF4 expression to detect human ASCs from peripheral blood [7] . A further defining characteristic of ASCs is their intracellular stock of immunoglobulins that can be readily measured by flow cytometry. The utility of these approaches is however limited as the assessment of these intracellular markers is incompatible with cell viability and most other molecular and functional assays. One strategy that circumvented this limitation was the development of reporter mice strains where fluorescent proteins, such as GFP, were incorporated into the *Blimp1* (*Prdm1*) locus [10, 11]. BLIMP-1 fulfils the criteria as an ASC marker as it is necessary for the differentiation of mouse ASCs from an early point and expressed in all ASCs in mouse and human [8, 10, 12, 13]. Although BLIMP-1-GFP mice have revolutionised our understanding of many aspects of ASC biology, including defining the differentiation process, gene regulatory networks and the trafficking of ASCs in the body, in normal and pathogenic circumstances, a strategy to define all ASCs in a non-transgenic model is also essential to be of the broadest possible utility.

The reports from Wilmore et al. [3] and Pracht et al. [4] provide examples of such approaches. Both studies use the standard combination of B220 (a relatively B-cell specific isoform of CD45) and CD138 (also called Syndecan-1) to loosely define ASCs in the mouse spleen and bone marrow [14]. Although these approaches capture many ASCs, it is well known, and reported by Wilmore et al. [3] and Pracht et al. [4] that a significant proportion of cells within these highly arbitrary gates lack BLIMP-1-GFP. To overcome this problem, Wilmore et al. [3] introduce Sca-1 (detecting Ly6A/E), a widely used marker of the hematopoietic progenitor compartment in the mouse, to refine ASC identification (Figure 1A, right panel). This gating strategy was validated using BLIMP-1-GFP fluorescence to define bona fide ASCs. The Sca-1^{+}CD138^{+} staining combination (in cells depleted for IgD^{+} mature B cells and some non-B cell lineages) efficiently identified a pure population of ASCs in the marrow and spleen, although a minority of BLIMP-1-

GFP$^+$ cells appeared to be excluded through a lower expression of CD138. It is also of note that the combination Sca-1 and CD138 was less effective at identifying BLIMP-1-GFP$^+$ cells in Peyer's patches. One should also be mindful that Sca-1 expression can be induced on many immune cell types following exposure to type I interferon or TNF [15, 16], suggesting that Sca-1 is not a faithful ASC marker in all circumstances.

Pracht et al. [4] adopt a similar strategy, but identify TACI as a marker of ASCs. TACI (also called TNFRSF13B) is a BAFFR family protein that is expressed widely in B cells, but whose expression level spikes in ASCs [17] (Figure 1A, right panel). Co-staining of TACI and CD138 identified a BLIMP-1-GFP$^+$ ASC population that approached purity in spleen and bone marrow, although Pracht et al. [4] did not determine the extent to which other BLIMP-1-GFP$^+$ ASCs occurred outside the TACI$^+$CD138$^+$ gate. Interestingly, the ASCs were heterogeneous for the B-cell markers B220 and CD19, with plasmablasts being enriched in the TACI$^+$CD138$^+$B220^{int}CD19^{int} fraction, while further down-regulation of B220 and CD19 was characteristic of maturation to the post-mitotic plasma cell phenotype. A similar finding was also reported by Wilmore et al. for B220 expression [3].

Although Sca-1 or TACI, in combination with CD138, represent excellent approaches to identify ASCs, other candidates have also been proposed (Figure 1B). Shi et al. used transcriptomic approaches to identify Ly6C, Ly6K, BCMA (Tnfrsf17), SLAMF7 and CD98 as additional murine ASC markers [6]. The anti-Ly6C mAb recognizes the products of two related genes *Ly6c1* and *Ly6c2* that are expressed very highly in ASCs ([18] and Figure 1B). Ly6K, while its expression is lower overall, has the advantage of specificity, as it is not expressed on other hematopoietic cells [19]. CD98 is an important amino acid transporter, also termed LAT1, which consists of heavy (SLC3A2) and light (SLC7A5) subunits. The increased expression of CD98 is most likely a reflection of the high metabolic requirements of ASCs [9]. Our analysis showed that virtually all of CD138$^+$BLIMP-1-GFP$^+$ ASCs expressed Ly6C and CD98 [6]. Finally, the gene encoding the T-cell co-stimulatory receptor CD28 is normally repressed by PAX5 in B cells [20]. Upon silencing of PAX5 during terminal differentiation, CD28 is re-expressed in ASCs [20, 21]. These studies collectively suggest that there are several candidates that with CD138 can act as ASC markers. The utility of the individual markers may depend on the experimental circumstances and potentially the availability of appropriate fluorescent conjugates.

The translation of these findings to human ASCs biology is problematic as many of the mouse genes listed, including *Ly6a, Ly6c1, Ly6c2* and *Ly6k* do not have direct human homologues [22] and CD28 is not expressed in human ASCs ([23] and Figure 1C). Moreover, the increased

expression of CD38 and CD27, used to identify human ASCs, does not occur in mouse ASCs. The genes encoding the components of CD98, *SLC3A2* and *SLC7A5*, are expressed in human ASCs (not shown), suggesting that this antigen warrants further investigation as a marker for human ASCs. TACI is found on human plasma cells, although expression is low in the bone marrow compared to its close relative BCMA [24]. SLAMF7 is highly expressed on human ASCs and multiple myeloma cells, but is also moderately expressed on many immune cells types [25, 26]. The expression of SLAMF7 on natural killer cells has been exploited to develop Elotuzumab, the first approved monoclonal Ab therapy for multiple myeloma that relies on antibody-dependent cell mediated cytotoxicity directed by natural killer cells against the tumor cells [26]. In contrast, high CD138 and the loss of CD45 and CD19 occur similarly to the mouse system, although again there are some reports of variation in CD138 expression and the timing of the loss of CD45 and CD19 [7, 27, 28].

The next level of discrimination is to identify ASC subpopulations, plasmablasts from plasma cells, and to find better markers for the long-lived plasma cells, as this population is likely to be a valuable therapeutic target. Plasmablasts can be efficiently distinguished from plasma cells in the mouse spleen using the BLIMP-1-GFP reporter, in particular the combination of intermediate GFP with MHCII or CXCR3 [6, 10]. The down-modulation of B220 and/or CD19 also broadly correlates with the ASC maturation steps, as proposed by Wilmore et al. [3] and Pracht et al. [4] in mice and recent studies on human samples [7, 27], but the resolution remains limited without using the BLIMP-1-GFP reporter strain. Moreover, whether plasmablasts elicited in distinct immunological contexts (for example T-dependent or T-independent stimuli) express distinct markers remains to be determined.

Despite their relative rarity, ASCs are critical players in the immune system. The findings of Wilmore et al. [3] and Pracht et al., [4] as well as the recent reports of the mouse ASC transcriptome [6] provide the field with a number of new approaches to define and quantify plasmablasts and plasma cells in a variety of contexts. Such improved strategies will support both an improved understanding of the biology of these cells as well as to facilitate the ongoing attempts to develop biologics that target ASCs clinically.

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Conflict of interest:

The authors declare no commercial or financial conflict of interest

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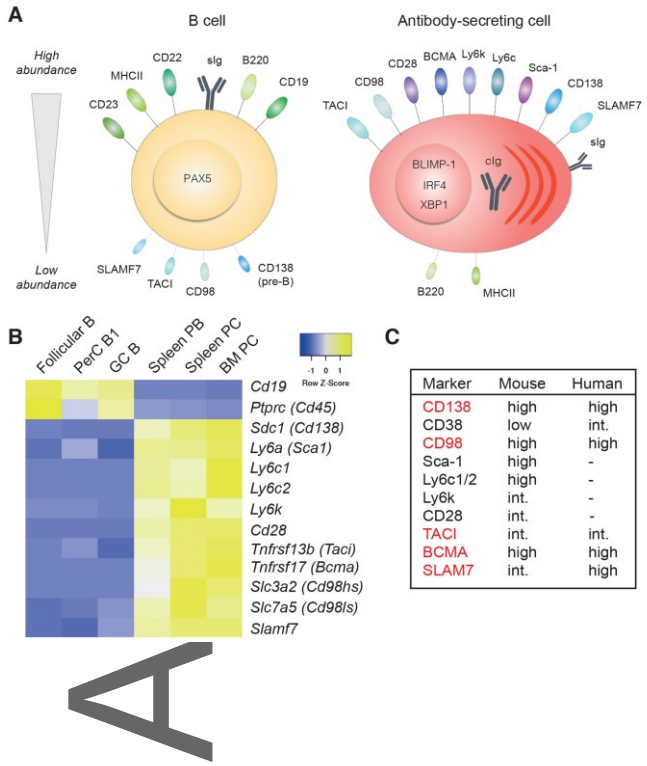
Figure legend

Markers of murine B cells and plasma cells. (**A**) B cells express the transcription factor PAX5 and surface molecules including CD19, CD22, MHCII, B220 and CD23 (left panel). In contrast, the high expression of the transcription factors BLIMP-1, XBP1 and IRF4, and the presence on the plasma membrane of CD138, TACI, BCMA, Sca-1, Ly6C, Ly6K, CD28, SLAMF7 and CD98 are

hallmarks of mouse plasma cells (right panel). The cell surface proteins with the highest abundance are depicted on the upper portion of the diagram, and those with lower abundance are shown on the lower portion. Lower expression of TACI, SLAMF7 and CD98 can be detected on B cells, and CD138 is transiently expressed at the pre-B cell stage (left panel). Similarly, B220 and MHCII expression is gradually lost during differentiation, being present on plasmablasts but not mature plasma cells (right panel). Finally, the cell membrane associated form of immunoglobulin is exclusively present on B-cell surface (sIg, left panel), whereas the secreted form vastly predominates in plasma cells, where high cytoplasmic stocks can be detected (cIg, right panel).

(**B**) Heat map showing the abundance of mRNA encoding the indicated surface markers in different populations of the B-cell lineage: follicular B cells, peritoneal cavity B1 cells (PerC B1), germinal center B cells (GC B), spleen plasmablasts (PB), spleen plasma cells (PC), bone marrow plasma cells (BM PC). Data in (**B**) were reported in [6].

(C) Comparison of the relative expression of the indicated markers in mouse and human ASCs. An arbitrary scale of high, intermediate (int), low or absent (-) is used. The human genome lacks direct homologues of murine Ly6A, Ly6C1/2, Ly6K.



Marker	Mouse	Human
CD138	high	high
CD38	low	int.
CD98	high	high
Sca-1	high	-
Ly6c1/2	high	-
Ly6k	int.	-
CD28	int.	-
TACI	int.	int.
BCMA	high	high
SLAM7	int.	high